PTO 97-2182

EUROPEAN PATENT NO.470,437
1992-02-12 (A1)

WATER-CONTAINING LIPOSOME SYSTEM
[Wasserhaltiges Liposomensystem]

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UNITED STATES PATENT AND TRADEMARK OFFICE
Washington, D.C. March 1997

Translated by: Diplomatic Language Services, Inc.

PUBLICATION COUNTRY	(19):	EUROPE
DOCUMENT NUMBER	(11):	470,437
DOCUMENT KIND	(12):	A1
	(13):	PATENT
PUBLICATION DATE	(43):	1992-02-12
PUBLICATION DATE	(45):	
APPLICATION NUMBER	(21):	91112377.6
APPLICATION DATE	(22):	1991-07-24
ADDITION TO	(61):	
INTERNATIONAL CLASSIFICATION	(51):	A61K 9/127
DOMESTIC CLASSIFICATION	(52):	
PRIORITY COUNTRY	(33):	DE; DE; DE
PRIORITY NUMBER	(31):	4024886; 4108902; 4122744
PRIORITY DATE	(32):	1990-08-06; 1991-03-19; 1991-07-10
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TITLE	(54):	WATER-CONTAINING LIPOSOME SYSTEM
FOREIGN TITLE	[54A]:	WASSERHALTIGES LIPOSOMENSYSTEM

The present invention concerns a water-containing liposome system according to the preamble of patent claim 1 as well as a procedure to manufacture such a liposome system. /2*

Phospholipid liposome systems for different applications are prior art. Accordingly, these systems are used in cosmetics or for manufacturing pharmaceutical products. The respective agents are encapsulated in the balls (vesicles) identified as liposomes, and the liposomes preferably contain an aqueous phase inside them in which the corresponding agent is then correspondingly dissolved, dispersed, or emulsified. On the outside, the liposomes are delimited by a lipid double membrane.

For example, EP A 03 09 519 and EP A 03 15 467 describe liposome systems that encapsulate the agent pentamidine and that are used as corresponding pharmaceutical products.

The prior art liposome systems frequently have the disadvantage that they tend to form an undesirable sediment after a short while.

The present invention is based on the problem of presenting a water-containing phospholipid liposome system that is particularly stable and hence does not form a sediment.

This problem is solved according to the invention by a liposome system with the characteristics of patent claim 1.

The water-containing liposome system according to the invention contains at least one phospholipid and at least one phospholipid charge carrier.

The liposome system according to the invention has a series of

*Numbers in the margin indicate pagination in the foreign text.

advantages. Accordingly, it has been determined that the liposome system according to the invention does not tend to form a sediment or deposits on walls even over extremely long storage periods of several months to years. In addition, the liposome system according to the invention is highly transparent and is not milky as is the case with prior art liposome systems. This makes it easy to test for foreign particles in the liposome system according to the invention since one only has to correspondingly check the clarity of the liposome dispersions. They can also be sterilized by filtration, which makes the liposome systems according to the invention especially suitable for pharmaceutical, cosmetic, or diabetic applications.

The described advantageous effects of the liposome systems according to the invention are based on the fact that the presence of the negative phospholipid charge carriers have a synergistic effect.

Particularly favorable results in regard to the cited advantages are attained by an embodiment of the liposome system according to the invention that contains at least one salt (preferably a sodium and/or ammonium salt) of phosphatidylglycerol and/or its derivatives as the phospholipid charge carrier. This is preferably the corresponding salt of dimyristoylphosphatidylglycerol and/or dipalmitoylphosphatidylglycerol.

With the liposome system according to the invention, the phosphatidylglycerol of the described embodiments in the form of a corresponding salt forms the preferred negative phospholipid charge carrier. It can basically be isolated and accordingly used after purification from any natural substance such as oil-bearing seeds,

rapeseed, sunflower seeds, etc. It is particularly suitable, however, when the cited salts of phosphatidylglycerol or the corresponding derivatives are isolated from soybeans so that a soy phosphatidylglycerol alkali salt, especially sodium or potassium salt, is the negative charge carrier in the liposome system according to the invention, or a soy phosphatidylglycerol derivative alkali salt, preferably sodium or potassium salt.

The mass ratio of at least one phospholipid to at least one negative phospholipid charge carrier is between 50:1 and 400:1 (and preferably 100:1-200:1). The cited small amounts of negative charge carrier are sufficient to give the phospholipid liposome system the described stability during storage and a high degree of transparency. An embodiment of the liposome system according to the invention that has a particularly long storage life and distribution of the liposomes contains phosphatidylcholine as the phospholipid. In particular, when the phosphatidylcholine is very pure, i.e., contains less than ca 10 weight percent impurities, a liposome system that preferably contains the described soy phosphatidylglycerol sodium potassium salt as the negative phospholipid charge carrier has the cited advantageous properties. In addition, such a special liposome system can be homogeneously /3 comminuted with substantially less effort and hence in ca one-half the time by high-pressure homogenization or ultrasound to a desired average partial diameter between 50 nm and 180 nm (and preferably 70 nm and 130 nm). Such a special liposome system can be easily sterilized by filtration; 0.2 μm filters are preferably used.

The phospholipid concentration of the liposome system according to

the invention is between 0.5 and 20 weight percent.

As stated, the liposome system according to the invention can be used for pharmaceutical and cosmetic purposes.

If the liposome system according to the invention is used for pharmaceutical purposes, there are two possibilities.

The first is to use a liposome system according to the invention in the form of an empty liposome system, i.e., the liposome system as such has a pharmaceutical effect. Such an empty liposome system is excellent for treating atherosclerosis, high blood lipid values, and hepatopathies of any genesis. Such a system preferably contains water and possibly alcohol and 5-15 weight percent of a mixture of phosphatidylcholine and a negative charge carrier in the cited mass ratio. In particular, such a pharmaceutical product can be injected when used.

The second possibility is to encapsulate an agent in the liposome system according to the invention. It was shown that such an encapsulated agent has an improved therapeutic effect in contrast to prior art preparations without impairing the goal of treatment. This effect is due to the fact that the agent encapsulated in the liposome system is released very evenly over a longer period during treatment so that undesired side effects do not arise or are at least substantially reduced.

The selection of the respective agent then revolves around the respective area of application. Accordingly, for example, pentamidine, pentamidine salts (especially pentamidine isethionate), and/or pentamidine derivatives can be dissolved and/or encapsulated, and the pharmaceutical product is preferably used for parenteral and especially

pulmonary treatment of Pneumocystis carinii pneumonia, African sleeping sickness, or kala azar.

It is particularly suitable when the cited agent is not added during the manufacture of the liposome system but is added directly before used. This can be done by mixing an aqueous liposome system with the agent as a dry substance, or initially dispersing a dry liposome system in water and then mixing it with the agent. Such a pharmaceutical product is then highly transparent. In certain cases, similar effects are attained by combining liposome preparations with the agent without the agent being encapsulated in the liposomes.

If, contrastingly, the liposome system according to the invention has doxorubicin as the agent x HCl, it can be used as a corresponding pharmaceutical product to treat cancer.

If, contrastingly, the liposome system according to the invention is used to treat viral illnesses, especially of the skin, it is useful to encapsulate a corresponding virucidal agent, preferably rosemary acid or dextran sulfate.

Furthermore, the corresponding prior art agents for treating cancer, AIDS, liver or viral illnesses can be encapsulated or added to the liposome system according to the invention.

The present invention also concerns a procedure to manufacture the described liposome system.

The procedure according to the invention to prepare the liposome system according to the invention is based on the approach of first dissolving or dispersing the used phospholipid, especially the described phosphatidylcholine or very pure phosphatidylcholine, together with the

phospholipid charge carrier, especially the soya phosphatidylglycerol sodium salt in an organic solvent. Then the solution or dispersion is evaporated to a lesser volume, and a corresponding amount of water is added to form the corresponding liposome system.

It is preferable to use ethanol, propanol 1, and/or propanol 2 as /4
the solvent in the described procedure according to the invention.

Depending on the used nontoxic organic solvent and its miscibility or compatibility with water, the initially prepared solution is evaporated down to different residual volumes. If, e.g., the cited alcohols are used as the nontoxic organic solvents, it is useful to concentrate the corresponding solution of the phospholipid with the negative phospholipid charge carrier to a residual volume between 3 and 30 percent by volume, and preferably 5-10 percent by volume. With organic solvents that are not miscible with water, it is recommendable to evaporate them until dry.

To prepare a liposome system using the procedure according to the invention that is distinguished by a particularly even, specific average liposome diameter, it is recommendable to subject the arising liposome system to high-pressure homogenization or ultrasound treatment after adding water. It is preferable to carry out these treatments until the arising liposomes have an average diameter of 50-180 nm.

In addition, the liposome system treated in this manner can be sterilized by filtration through a 0.2 μm filter.

The liposome systems created in this manner can then either be put in corresponding ampules ready for use or carefully dried (especially freeze-dried) after the addition of suitable adjuvants, especially

carbohydrates, to yield powdered liposome systems that form the desired ready-to-use vesicles by adding a suitable amount of water without time-consuming stirring or other mixing being necessary.

There are again two possibilities for preparing the embodiments of the liposome system according to the invention that has the cited agents.

The first possibility is to directly add the agent together with the phospholipid and phospholipid charge carrier to the organic solvent at the beginning of the procedure according to the invention. In a variation of this procedure, the used phospholipid is mixed with the agent dissolved, dispersed or emulsified in a nonaqueous solvent, and then after careful drying, the mixed phospholipid is dissolved together with the phospholipid charge carrier in an organic solvent that possibly is different from the first solvent. Then the organic solvent as described above is concentrated, and the water is subsequently added to form the agent-liposome system whereby the agent can then be encapsulated. This procedure is particularly good for those instances in which the agent is stable in storage.

The second possibility (particularly preferred when the agent is not soluble in the initially used organic solvent but is more soluble in water) is to make the aqueous liposome system in the manner described above and then add the water and agent together.

A variation of the described procedure that is particularly preferred when the agent has a limited storage life uses a powdered, dried liposome system. The water and agent are added during redispersing so that the agent only comes into contact with the liposome

system directly before using the product.

To exclude undesirable byproducts, the procedure according to the invention is preferably carried out under inert gas.

Advantageous developments of the procedure according to the invention are listed in the subclaims.

The procedure according to the invention will be further explained in the following with reference to exemplary embodiments.

Example 1

Preparation of an Empty Liposome System

99.5 g highly pure phosphatidylcholine (i.e., less than 10 weight percent impurities) and 0.5 g soy phosphatidylglycerol sodium salt (PG) are dissolved in 500 ml ethanol DAB 9 and then dried in a vacuum. The obtained phospholipid mixture is dispersed by agitation under an inert gas in water for injection purposes at 1,000 ml. An average particle diameter of < 100 is then produced in five cycles through a high-pressure homogenizer. The arising liposome system was then filtered through a 0.2 μm filter under sterile conditions and poured into 10.0 ml am- /5
pules under inert gas. The phosphatidylcholine/soy PG sodium liposome system prepared according to Example 1 has the following characteristics:

Phosphlipid content:	10% (m/V)
Appearance:	Transparent, slightly opalescent liquid
pH:	6.1
Viscosity:	2.6 mPa/s
Osmotic pressure:	0.49 (% NaCl)
Transmission (660 nm):	75%
Ave. particle diameter (laser light scatter):	75 nm
Sterility:	Corresponds to sterility test, DAB 9
Endotoxin content (limulus test):	Corresponds to requirements of DAB 9
Electronmicroscopic characterization (cryofixation):	40-100 mn unilamellar liposomes, rare bilamellar liposomes

Given its composition, this product can be used in the following applications: atherosclerosis, increased blood lipid values, hepatopathies of any origin.

Example 2

500 g phospholipid mixture consisting of 497.5 g highly pure phosphatidylcholine (i.e., less than 10 weight percent impurities) and 2.5 g soy PG sodium salt is prepared according to Example 1 and then dispersed in 6.5 l water for injection purposes by agitation and under inert gas. Water for injection purposes is added to the preparation up to 8.0 l. In a separate batch container, 2 kg maltose in 1.5 l water for injection purposes is dissolved while heating to 70°C. By passing through several cycles in a high-pressure homogenizer (APV Gaulin, type LAB 60), the phospholipid system is brought to an average particle diameter of 56 nm and mixed with the maltose solution while agitating under inert gas, water for injection purposes is added to 10.0 l, and the preparation is sterilized by filtration, poured into ampules under

aseptic conditions, and freeze-dried. The lyophilizate that arose after freeze-drying has the following characteristics:

Appearance:	Crystalline, slightly yellow dry powder
Residual moisture according to Karl Fischer's method:	< 0.7%
Phospholipid content:	500 mg/vial
Sterility:	Corresponds to sterility test, DAB 9
Endotoxin content (limulus test):	Corresponds to requirements of DAB 9

After redispersing the lyophilizate with 8.3 ml water for injection purposes, a liposome system was obtained with the following properties:

Appearance:	Transparent, slightly opalescent liquid
pH	6.5
Viscosity:	2.7 mPa/s
Transmission (660 nm):	72%
Ave. particle diameter (laser light scatter method):	60 nm

The phospholipid system prepared according to Example 1 and the lyopholizate from Example 2 can be used for the following purposes: atherosclerosis, increased blood lipid values, hepatopathies of any cause. In contrast to the aqueous liposome system from Example 1, the lyopholizate prepared according to Example 2 has the advantage of greater stability.

The phospholipid mixture prepared according to Example 1 consisting of phosphatidylcholine and soy PG sodium salt can be used to prepare empty, sterile-filterable phosphatidylcholine liposome systems/6 (Examples 1 and 2) and sterile liposome systems with an agent (Examples 3-5).

Example 3

10 g of the invented phospholipid mixture according to Example 1 was dissolved together with 0.1 g propidium iodide (DNA marker) in ethanol and dispersed in 100 ml water for injection in a vacuum under inert gas and cooled. Then the preparation was subjected to ultrasonic treatment under inert gas and cooled until an average liposome particle diameter of 80 nm (laser light scatter) was attained. The liposome system was then sterilized by filtration through a 0.2 μm filter, and one-half was poured into brown 1.0 ml ampules under inert gas. The amount of liposome-bonded propidium iodide in the sterile liposome system with propidium iodide was determined by dialysis (Dianorm® device, cellulose triacetate membrane NMGT 20000). The amount of liposome-bonded propidium iodide was 29%. In the second half sterilized by filtration, the amount of propidium iodide not bound to the liposomes was separated by ultrafiltration via a cellulose triacetate membrane NMGT 20000, and the liposome dispersion was again sterilized by filtration through a 0.2 μm filter and poured into 1.0 μm brown ampules under inert gas. The obtained liposome dispersion has the following properties:

Phospholipid content:	100 mg/ml
Propidium iodide content:	0.285 mg/ml
pH:	7.2
Viscosity:	1.7 mPa/s
Ave. particle diameter	129 nm

Example 4

18.4 g of the phospholipid mixture described in Example 1 was dissolved together with 0.92 g quinoline yellow in ethanol, dried in a

vacuum, dispersed with water for injection at 200 ml, and then treated with ultrasound while cooling. The obtained liposome system was then sterilized by filtration and poured under aseptic conditions into 5.0 ml injection bottles. The liposome dispersion sterilized by filtration has the following properties:

Appearance:	Transparent, slightly opalescent liquid
pH	6.4
Ave. particle diameter	75 nm
Transmission (660 nm):	33%
Sterility:	Corresponds to sterility test, DAB 9
Quinoline yellow, liposome-bound:	1.38 mg/ml
Quinoline yellow, not liposome-bound:	3.2 mg/ml

To determine the amount of liposome-bound quinoline yellow, the quinoline yellow component that was not liposome-bound was separated by ultrafiltration through a cellulose triacetate membrane (NMGT 20000), and the amount of quinoline yellow in the liposome dispersion and in the filtrate was measured photometrically.

The phospholipid mixture transformed into a dry powder corresponding to Example 2 is ready to use for preparations of liposomes containing active water-soluble substances.

Example 5

Sterile dry powder corresponding to the 500 mg phospholipid mixture described in Example 1 and 2,000 mg carrier were dispersed with 5.0 ml doxorubicin HCl solution (10.0 mg doxorubicin HCl). The arising liposome redispersant (6.8 ml) with doxorubicin HCl contains 73.5 mg/ml phospholipids and an overall doxorubicin HCl content of 0.735 mg/ml. The amount of liposome-bound doxorubicin HCl was found to be 0.58 mg/ml

and corresponds to an inclusion/encapsulation rate of ca 78%. /7

The amount of liposome-bound doxorubicin HCl was calculated by dialysis using liposomates, 5.0 ml, for 5 h.

Example 6

Preparation of an Empty Liposome System

100 g highly pure phosphatidylcholine, i.e., less than 10 weight percent impurities, and 0.502 g soy phosphatidylglycerol sodium salt (PG) were dissolved in 500 ml ethanol (DAB 9), and then the dry substance content was adjusted to 92 weight percent in a vacuum. The obtained phospholipid mixture consisting of 91.54 weight percent phosphatidylcholine, 0.46 weight percent soy phosphatidylglycerol sodium salt, 6 weight percent ethanol, and 2 weight percent water was dispersed in water for injection up to 1,000 ml while stirring under inert gas and then processed in a high-pressure homogenizer in five cycles to yield an average particle diameter of $<$ 100 nm. The arising liposome system was then filtered under sterile conditions through a 0.2 μm filter and poured into 10.0 ml ampules under inert gas. The phosphatidylcholine/ soy PG sodium liposome system prepared according to Example 6 has the following properties:

Phosphatidylcholine content: . .	10% (m/V)
Appearance:	Transparent, slightly opalescent liquid
pH:	6.1
Viscosity:	2.6 mPa/s
Osmotic pressure:	0.49 (% NaCl)
Transmission (660 nm):	75%
Ave. particle diameter (laser light scatter):	75 nm
Sterility:	Corresponds to sterility test, DAB 9
Endotoxin content (limulus test):	Corresponds to requirements of DAB 9

Given its composition, this product can be used in the following applications: atherosclerosis, high blood lipid values, and hepatopathies of any genesis.

Example 7

Preparation of an Empty Liposome System

100 g highly pure phosphatidylcholine, i.e., less than 10 weight percent impurities, and 0.502 g soy phosphatidylglycerol sodium salt (PG) were dissolved in 500 ml ethanol DAB 9, and then the dry substance content was adjusted to 92 weight percent in a vacuum. The obtained phospholipid mixture consisting of 91.54 weight percent phosphatidylcholine, 0.46 weight percent soy phosphatidylglycerol sodium salt, 6 weight percent ethanol, and 2 weight percent water was dispersed in water for injection while stirring under inert gas up to 8333 ml and then processed in a high-pressure homogenizer at 500 bar in five cycles to yield an average particle diameter of < 100 nm. The arising liposome system was then filtered under sterile conditions through a 0.2 μm filter and poured into 10.0 ml ampules under inert gas. The phosphatidylcholine/soy PG sodium liposome system prepared according to

Example 7 has the following properties:

Phosphatidylcholine content: . .	1.2% (m/V)
Appearance:	Transparent, slightly opalescent liquid
pH:	6.19
Viscosity:	1.4 mPa/s
Transmission (660 nm):	82%
Ave. particle diameter (laser light scatter):	58 nm
Sterility:	Corresponds to sterility test, DAB 9
Endotoxin content (limulus test):	Corresponds to requirements of DAB 9

Patent Claims /8

1. Aqueous liposome system that may contain at least one phospholipid in addition to a nontoxic organic solvent, characterized by the fact that the liposome system comprises at least one phospholipid charge carrier in addition to the phospholipid.

2. Liposome system according to claim 1, characterized by the fact that it has at least one salt, preferably sodium and/or ammonium salt, of phosphatidylglycerol and/or its derivatives.

3. Liposome system according to claim 2, characterized by the fact that it contains the salt of dimyristoylphosphatidylglycerol and/or dipalmitoylphosphatidylglycerol as the charge carrier.

4. Liposome system according to claims 2 or 3, characterized by the fact that the phosphatidylglycerol is a soy phosphatidylglycerol.

5. Liposome system according to one of the prior claims, characterized by the fact that the mass ratio of the phospholipid to the phospholipid charge carrier is 50:1-400:1, and preferably 100:1-200:1.

6. Liposome system according to one of the prior claims, characterized by the fact that it contains between 0.5 and 20 weight

percent phospholipid.

7. Liposome system according to one of the prior claims, characterized by the fact that it contains phosphatidylcholine as the phospholipid.

8. Liposome system according to claim 7, characterized by the fact that the phosphatidylcholine is highly pure phosphatidylcholine containing less than 10 weight percent impurities.

9. Liposome system according to one of the prior claims, characterized by the fact that it contains at least one pharmaceutically effective substance.

10. Liposome system according to claim 9, characterized by the fact that the effective substance is an agent to treat cancer, AIDS, liver and viral illnesses, or pneumocystis carinii pneumonia.

11. Procedure to prepare a liposome system according to one of claims 1-10, characterized by the fact that the phospholipid together with the phospholipid charge carrier are initially dissolved or dispersed in an organic solvent, then the solution or dispersion is concentrated by evaporation, and water is then added to form the liposome system.

12. Procedure according to claim 11, characterized by the fact that ethanol, propanol 1, and/or propanol 2 is used as the organic solvent.

13. Procedure according to claims 11 or 12, characterized by the fact that the solution or dispersion is concentrated until the residual volume of the solvent is 0-30 %/vol. and preferably 5-10 %/vol.

14. Procedure according to claims 11-13, characterized by the fact that the liposome system that arises after adding the water is subjected

to a high-pressure homogenization or ultrasonic treatment.

15. Procedure according to claim 14, characterized by the fact that the high-pressure homogenization or ultrasonic treatment is carried out until the formed liposome has an average diameter of 50-180 nm.

16. Procedure according to claims 11-15, characterized by the fact that the liposome system is filtered through a 0.2 μm filter.

17. Procedure according to claims 11-16, characterized by the fact that the liposome system formed by the addition of water is carefully dried, especially freeze-dried, after the addition of a suitable adjuvant, especially a hydrocarbon. /9

18. Procedure to prepare a liposome system with an active ingredient according to one of claims 9-17, characterized by the fact that the agent is dissolved, emulsified, or dispersed together with the phospholipid and the phospholipid charge carrier in the organic solvent.

19. Procedure to prepare a liposome system with an agent according to claim 17, characterized by the fact that the dried liposome system is added to water to which is added at least one pharmaceutically effective substance.